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7/29/02

Docket: 14256

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Peter Hagerlid		
Application No.:	09/936,932	Examiner:	
Filing Date:	September 19, 2001	Group Art Unit:	1743
Title:	LIQUID DISPENSING APPARATUS		

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Janet M MacLeod
(Signature)

Sir:

Enclosed is a certified copy of Great Britain Application No. 9906477.6 filed March 19, 1999, from which the present application claims priority under 35 USC § 119.

Respectfully submitted,

DORSEY & WHITNEY LLP

Date: July 11, 2002

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Enclosure

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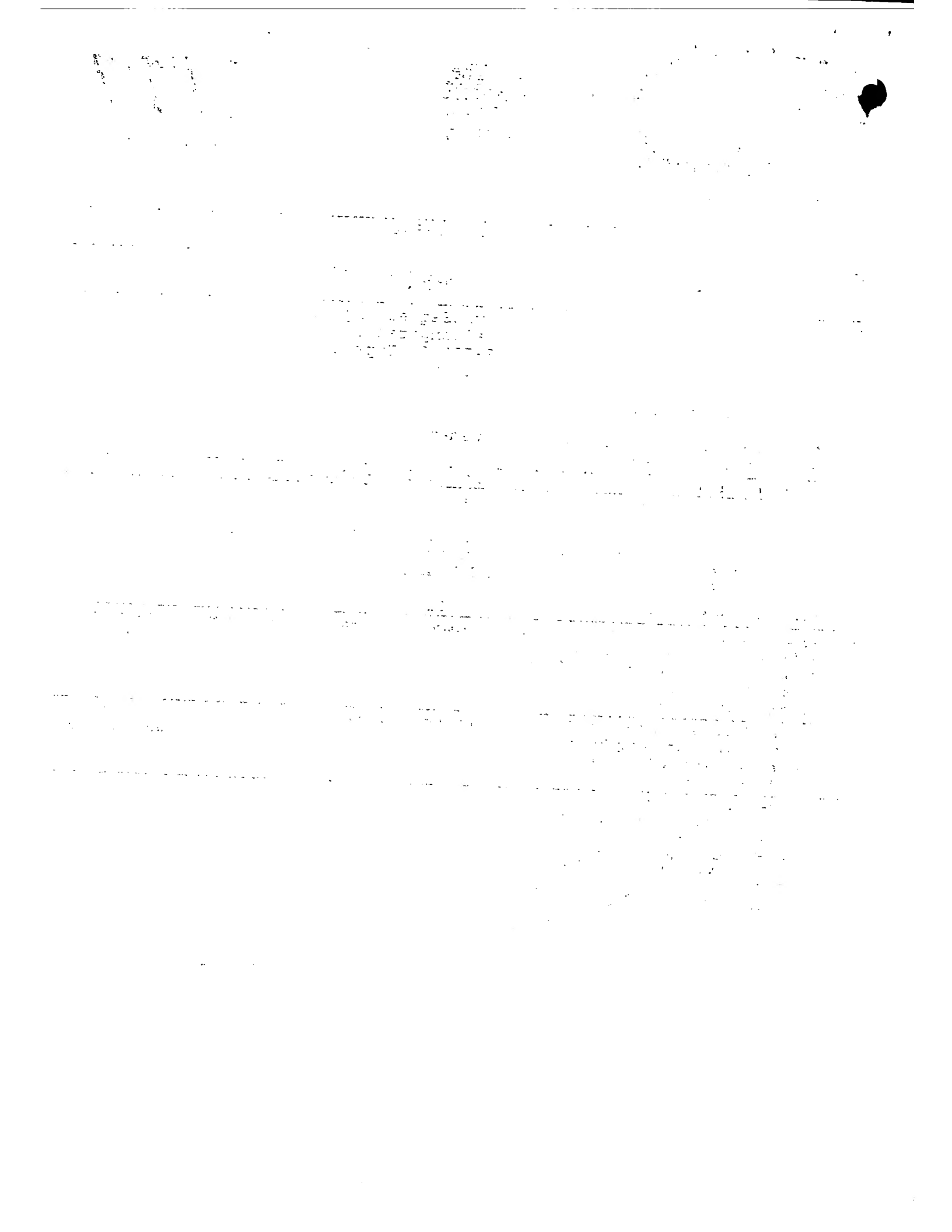

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1. **Your reference** — 38.46.68343

2. **Patent application number** *(The Patent Office will fill in this part)* — **9906477.6**

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)* — PyroSequencing AB
Vallongatan 1
S-752 28 Uppsala
Sweden

Patents ADP number *(if you know it)* — 7459605001

If the applicant is a corporate body, give country/state of incorporation — Sweden

4. **Title of the invention** — Liquid dispensing apparatus

5. **Name of your agent** *(if you have one)* — Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)* — 166001

6. **If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. **If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**

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a) any applicant named in part 3 is not an inventor, or
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11. **I/We request the grant of a patent on the basis of this application.**

Signature **Date** 19 March 1999

12. **Name and daytime telephone number of person to contact in the United Kingdom**

A.J. Piésold
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Liquid Dispensing Apparatus

This invention relates to liquid dispensing apparatus and in particular to liquid dispensing apparatus which are able to dispense small volumes of liquid.

In certain applications it is desirable to be able to dispense very small volumes of liquid from a container, for example in the range 50-500 nanolitres (nl). Drops of this size cannot normally be produced by pumping liquid out of a capillary since surface tension will hold the drop to the tip of the capillary until it is sufficiently large that its weight overcomes this surface tension. This does not happen until the volume of the drop is of the order of 10 to 50 microlitres - i.e. 2 to 3 orders of magnitude greater than the range of interest.

Devices are available which overcome this limitation by forcing a measured amount of liquid through the tip of a nozzle using a piezo-electric actuator acting on the liquid. However such systems are expensive and may only be used with a single type of liquid at a time - thus multiplying the cost where several different liquids need to be dispensed.

It is an object of the present invention to provide an improved apparatus and when viewed from a first aspect the invention provides a liquid dispensing apparatus comprising a liquid reservoir, a nozzle and driving means for forcing liquid through said nozzle, said driving means comprising means for generating a pulse of gas which impinges upon liquid in the reservoir so as to force liquid through the nozzle.

Thus it will be seen that in accordance with the invention liquid may be dispensed by forcing it through the nozzle by means of a pulse of gas. The volume of liquid dispensed may be controlled by adjusting the

amplitude and duration of the gas pulse. It has been found that this enables accurate control of the volume dispersed, down to the order of 50-500 nl in the preferred embodiment. Furthermore as a gas is used as the medium for transmitting force to the liquid, it is not necessary for any part of the driving means to be in contact with the liquid to be dispensed. This has clear advantages both from the point of view of preventing contamination of the liquid and also the reduced necessity to clean the apparatus.

As the driving impulse is applied to liquid in the reservoir, it is not necessary to have any part of the driving means arranged in the vicinity of the nozzle. This means that the nozzle may be a simple capillary tube and may even be disposable. The nozzle could comprise a separate part removable from the rest of the apparatus. Preferably however the nozzle is an integral part of the apparatus. In a particularly preferred embodiment one or both of the nozzle and the liquid reservoir are moulded from a suitable plastics material, thereby allowing them to be made disposable. More preferably the nozzle and liquid reservoir are both moulded from suitable plastics, most preferably integrally with each other, to form a cartridge. Alternatively such a cartridge or just the nozzle could be made from one or more other suitable materials such as silica or stainless steel.

Cartridges of the kind described above may be provided singly, but preferably a plurality are provided in a cassette. Such an arrangement is novel and advantageous in itself and thus when viewed from a second aspect the present invention provides a cassette comprising a plurality of cartridges for dispensing liquid therefrom, each cartridge comprising a nozzle and a liquid reservoir integrally formed with the nozzle, wherein said nozzle comprises a capillary tube.

The invention makes it possible, at least in some

preferred embodiments, to provide cartridges pre-filled with the necessary liquid and having the required capillary nozzle formed integrally therewith. All that is necessary then is simply to place the liquid reservoir of the cartridge or each cartridge of a cassette of cartridges in gaseous communication with a means to generate gas pulses, in order to form a liquid dispensing apparatus in accordance with the first aspect of the invention. This is extremely convenient to a user who thus does not need to be concerned with filling reservoirs with liquid, cleaning the nozzle etc. Indeed in at least preferred embodiments of this aspect of the invention, contact with the liquid to be dispensed may be completely avoided.

Alternatively the cartridges may be filled with a suitable liquid prior to use, e.g. a lyophilised reagent which is dissolved in water when required. The preferred embodiments of the invention in which the liquid reservoir is adjacent the nozzle are advantageous in this context since just the required amount of reagent can be made up as required without extra being required to accommodate dead space in pipes etc.

Cassettes of cartridges in accordance with the invention as described above are useful in many applications including but not limited to immuno assays, cell assay, drug screening and they are particularly useful for sequencing DNA using the method often referred to as "Sequencing By Synthesis" as they allow for example a cassette having cartridges containing each of the four nucleotides required for DNA sequencing. Preferably therefore the cartridges respectively contain one or more nucleotides. The ability to provide a plurality of cartridges in a single cassette further allows a complete set of reagents required for a sequencing programme to be provided. Thus preferably further cartridges of the cassette respectively contain one or more enzymes, more preferably selected from a

group comprising polymerase, luciferase, adenosine triphosphate (ATP) sulfurylase, and a nucleotide-degrading enzyme such as apyrase. Such a set of reagents is particularly useful for the method of genetic sequencing set out in WO 98/13523. Indeed it will be appreciated that the invention extends to a method of genetic sequencing using a liquid dispensing apparatus as herein described.

As discussed above, cartridges of the kind described above may be filled by a user with the appropriate liquid as required. Alternatively the cartridge or each cartridge of a cassette is pre-filled with the appropriate liquid and sealed, the seal being broken upon mounting the cartridge into an apparatus which includes the gas pulse generation means. Preferably the seal is broken by the action of mounting the cartridge into the apparatus. Advantageously a conduit for establishing gaseous communication with the interior of the liquid reservoir of the cartridge breaks the seal.

It will be seen from the above that a sealed cartridge containing a liquid to be dispensed by means of apparatus in accordance with the first aspect of the invention is in itself not only novel, but particularly convenient for a user and thus when viewed from a further aspect the present invention provides a cartridge comprising a liquid reservoir having a predetermined liquid received therein, said reservoir being closed at one end thereof by frangible sealing means, and a nozzle attached to or integrally formed with said reservoir and in fluid communication therewith.

As an alternative it will be seen that the invention extends to a cartridge for use in a liquid dispensing apparatus as hereinbefore described comprising a liquid reservoir, and a nozzle attached to or integrally formed with said reservoir and in fluid

communication therewith, in combination with a reagent, preferably a nucleotide or enzyme.

The reagent e.g. nucleotide or enzyme could be in a suitable liquid form or could be lyophilised. In the latter case a readily available diluent such as water could be used, or a suitable diluent could instead also be provided as part of the combination.

The frangible sealing means preferably comprises a foil membrane e.g. of aluminium covering an opening onto the reservoir. This is a cost-effective way of retaining liquid in the reservoir without it becoming contaminated, whilst at the same time being relatively easy to pierce - e.g. by a gas nozzle associated with the gas pulse generation means.

The predetermined liquid preferably comprises a nucleotide or an enzyme.

Where an enzyme is provided, either as the predetermined liquid in a pre-filled cartridge or as a separate reagent to be added by a user, the enzyme is preferably selected from a group comprising polymerase, luciferase, adenosine triphosphate (ATP) sulfurylase, and a nucleotide-degrading enzyme such as apyrase.

The bore of the nozzle or nozzles is preferably within the range 0.05 to 0.2 mm, most preferably about 0.1 mm. The length of the nozzle is preferably in the range 1 to 15 mm, most preferably 5 mm.

The means for generating the gas pulses may comprise any suitable means - e.g. a bellows arranged to undergo a rapid reduction in volume. Preferably however the pulse generation means comprises a source of pressurised gas which may be selectively placed into communication with the liquid reservoir. Such arrangements are especially beneficial since a single source of pressurised gas may be used to drive a plurality of liquid reservoirs, thereby giving a significant saving in cost over arrangements where driving means are individually provided for each of a

number of nozzles. Means for selectively placing the liquid reservoir into communication with the source of gas may be provided for each of such liquid reservoirs or two or more of them may be associated with a single selection means so that their contents are dispensed simultaneously.

Preferably one or more valves is provided to effect said selective communication and in the preferred embodiment an electromagnetic valve is used. This is particularly advantageous since electromagnetic valves can be operated very quickly and accurately with little mechanical wear.

Where, as is preferred, the apparatus for dispensing liquid comprises gas pulse generation means in the form of means for selectively communicating a source of pressurised gas with the liquid reservoir, the source of pressurised gas may be comprised within the apparatus. Most preferably the source of pressurised gas comprises a compressor supplying a pressure reservoir. Alternatively, the apparatus may comprise a gas inlet for connection to an external source of pressurised gas. In either case the apparatus of the invention preferably comprises means to regulate the pressure of the incoming gas thereby allowing the amplitude of the pulses generated to be accurately controlled and thus the volume of liquid to be accurately controlled as a consequence. The pressure regulation means may comprise a mechanical release valve or the like. Preferably however such means comprises an electronic pressure sensor which is used to monitor the pressure in the reservoir or gas inlet and means for increasing or decreasing said pressure in response to the signal from the pressure sensor to maintain the pressure within a predetermined range.

The gas pulse generation means preferably generates pressure pulses having an amplitude in the range 200 to 1000 millibars, with a pulse width in the range 1-1000

milliseconds. This enables volumes in the range 50 nl to 10000 nl (10μl) to be dispensed.

A preferred embodiment of the present invention will now be described, by way of example only, with reference to the accompanying Figure which is a schematic view of a cassette of cartridges and liquid dispensing apparatus in accordance with the invention.

Turning to the Figure there may be seen a liquid dispensing apparatus comprising a cartridge cassette designated generally by the reference numeral 1. This cassette contains eight cartridges 2, three of which are visible in the drawing. Each cartridge 2 is partly filled with a reagent used for genetic sequencing. The volume of the cartridges is approximately 2 ml. Different reagents are provided in each cartridge 2. Specifically, four of the cartridges 2 contain the deoxynucleotide triphosphate of one each of the four bases cytosine, guanine, adenosine and thymine. Four further cartridges 2 contain polymerase, luciferase, apyrase and ATP sulfurylase. Equally however some or all of the cartridges could have the same contents.

At the end of each cartridge 2 there is a capillary nozzle 6. This nozzle has a bore of 0.1 mm. In the depicted embodiment the nozzle 6 is integrally moulded with the rest of the cartridge 2 from a polymer. However the cartridge could be formed with means to receive a separate nozzle.

The cassette 1 has an outer case 8 which protects the cartridges 2 from contamination by the user handling them and conversely protects the user from having to come into contact with the liquid being dispensed or the very fine, i.e. sharp nozzles 6. Apertures 10 are provided in the base of the cassette casing 8 which are aligned with the nozzles 6 to allow the liquid through.

At the upper end of the cassette 1 a thin metal foil 16 is provided over the tops of the cartridges 2 to seal them. The foil 16 extends across the open upper

end of the cassette 1 formed by the side walls of the casing 8, but slightly downwardly set from the top edge so as to leave un upstanding lip around the top of the foil 16.

The cassette 1 is installed in a carriage 18 which can be moved laterally in both directions over a Micro Titre Plate (MTP) 12. The MTP has 96 wells 14, three of which are shown. The cassette 1 is actually received in a downwardly tapering chamber 20, so as to rest on an apertured plate 22, the apertures of which are in alignment with the apertures 10 in the cassette and, when the carriage 18 is properly positioned, also with the wells 14 of the MTP 12.

The cassette 1 is retained in the chamber 20 by means of a hinged lid 3, having a rubber seal 24 on its underside which is a tight fit inside the lip at the top edge of cassette casing 8. The seal 24 is arranged also to ensure that the cartridges 2 are sealed with respect to one another as well as with respect to the lid 3.

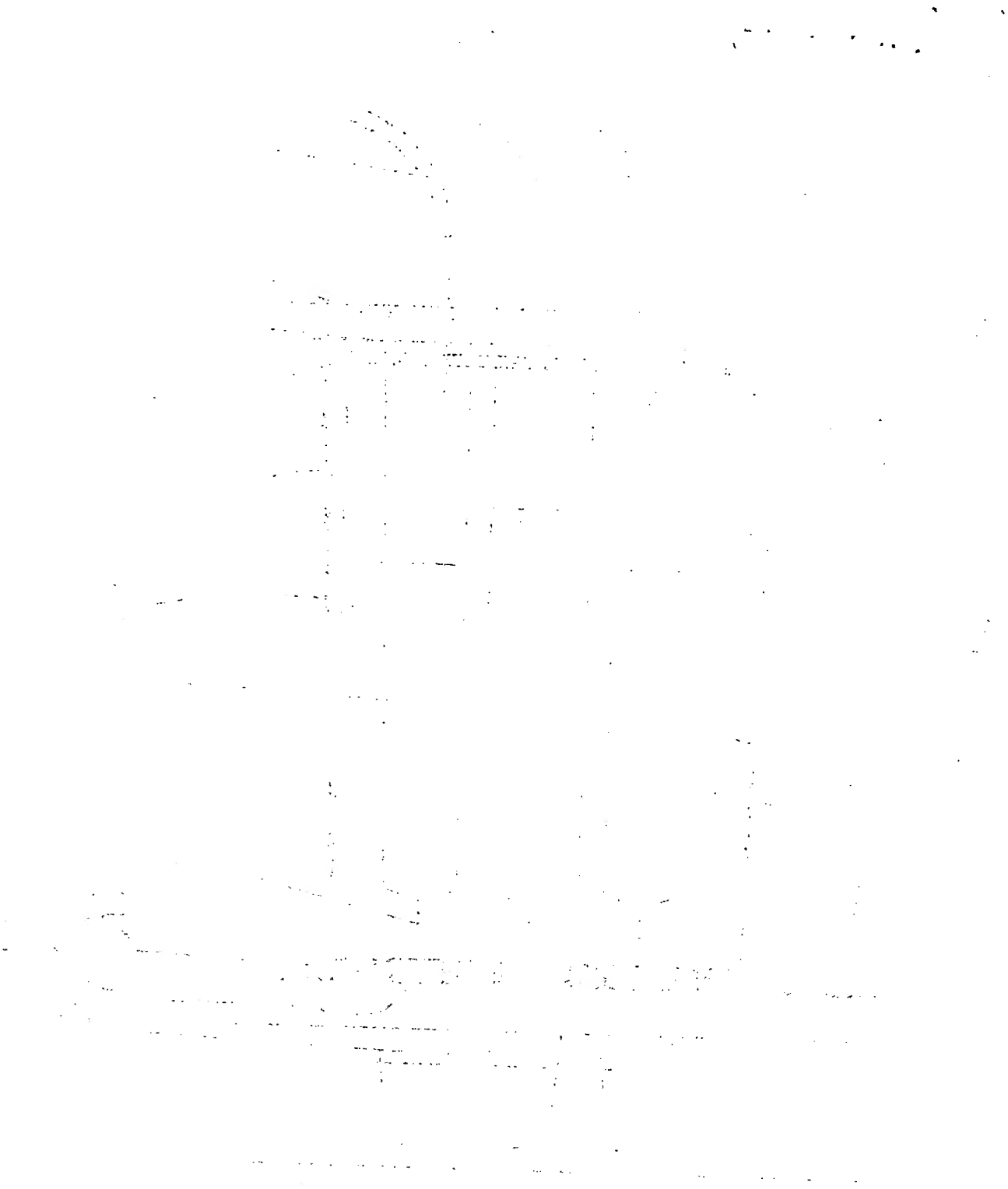
Mounted in the lid 3 is an array of punching cannulae 28, one for each of the cartridges 2. These cannulae 28 have sharp tips and so as the lid 3 is hinged downwardly onto the cassette 1 in the chamber 20, the cannulae 28 pierce the foil seal 28. The other ends of the cannulae are each connected by means of a pipe 26 to an electromagnetic valve (not shown) which selectively connects the pipe 26 to a source of pressurised gas for a predetermined time to produce a pulse of gas. The pressurised gas is generated by a compressor type EC Genius/M 202 2305 EU.SV.C which is commercially available from Fini, Zola, Predosa, Bo - Italy.

The pulse of gas forces a measured amount of reagent 4 out of the cartridge 2, through the nozzle 6 and into the corresponding well 14 on the MTP 12. A 500 millibar pulse, for 10 milliseconds dispenses approximately 200 nanolitres of reagent into the well

14. Reagent is only dispensed from one of the cartridges 2 at a time although simultaneous dispensing is also possible and this could be useful e.g. where a plate having a greater number of wells was used with an interleaved series of wells used so that dispensing into the wells of several distinct series can be carried out without moving the carriage 18.

Once the reagents in the cartridges have been used, the cassette may simply be removed by opening the lid 3 and replaced with a new one, the fresh seals being broken by the cannulae 28. Since neither the cannulae 28 nor any other permanent part of the apparatus comes into contact with the reagents at any stage, there is little chance of contamination and it is not necessary to clean the apparatus between uses, even if different reagents are being used.

Although an embodiment of the invention which has a cassette comprising several cartridges has been described, it will be understood by those skilled in the art that the invention is not limited to using several cartridges and that a single cartridge reservoir could be used instead. Furthermore, the liquid reservoir need not be removable and could instead be an integral part of the apparatus.



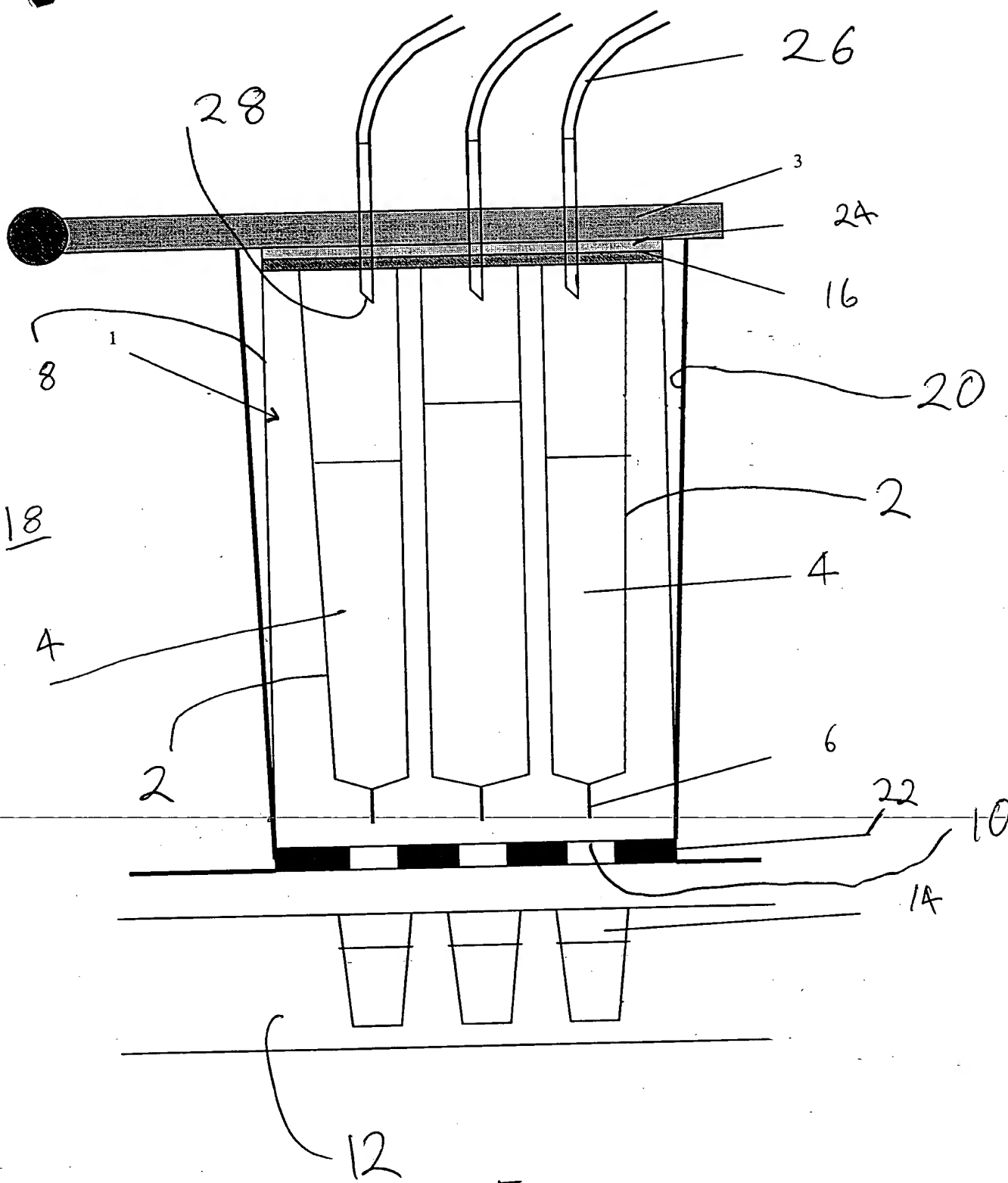


Fig.